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CYANONEWS

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CYANONEWS - a newsletter intended to provide cyanobacteriologists with a forum for rapid informal communication, unavailable through journals. Everything you read in this newsletter is contributed by readers like yourself. Published occasionally, about three times per year.

SUBSCRIPTIONS - $10 or equivalent/year. (See last page)

CONTRIBUTIONS - Expected every couple of years: a new result, an upcoming meeting or a summary of a past meeting, a post-doctoral opening, a new publication, a request for strains, a change of life... something. See last page for addresses you can send news to.

HOW TO FIND OUT MORE ABOUT SOMETHING YOU READ HERE - Contact the person whose name is capitalized in the news item. Addresses are given at the end of the issue. Also, a Directory of Cyanobacteriologists is distributed every two years. If you need one, write to Jeff Elhai (see last page of newsletter).

INSTRUCTIONS TO AUTHORS - Send news.

BULLETINBOARD*BULLETINBOARD*BULLETINBOARD*BULLETINBOARD*BULLETINBOA

Several people have complained justifiably that this newsletter contains a disproportionate amount of news about molecular biology as compared to WHOLE-ORGANISM CYANOBACTERIOLOGY. Needless to say, whatever is contributed is what appears in these pages. However, there very well may be an unhealthy cycle operating: ecologists tend not to contribute to a newsletter that appears to ignore their concerns. Solution: Break the cycle! If your favorite area of research isn't receiving much ink, send in some news! (And encourage others in the area to do the same).

A much updated and corrected DIRECTORY OF EMAIL ADDRESSES of cyanobacteriologists has been compiled and is available. Contact:

Jeff Elhai, Cyano@MSU.Bitnet or Cyano@MSU.Edu or Cyano@MSU

An INTERNATIONAL SYMBIOSIS CONGRESS will be held in Jerusalem, Israel, 17-22 Nov 1991. It has been organized around topics rather than specific symbioses, hoping to encourage discussions amongst workers focusing on different systems. The topics include nutritional interactions and carbon and nitrogen metabolism. The registration fee is US $290 or $180 (students). Accomodations range from US $15 to $67 per person per night. Contact:

Margalith Galun, Symbiosis Research Laboratory, Dept. of Botany, Tel-Aviv University, Tel-Aviv 69978, ISRAEL (Tel) 972-3-5459163 (FAX) 072-3-6426211 (EMail) EEEM@Taunos.Bitnet

The SIXTH INTERNATIONAL SYMPOSIUM ON MOLECULAR PLANT-MICROBE INTERACTIONS is scheduled for 11-17 July 1992, in Seattle, Washington, U.S.A. Contact:

Eugene Nester, Dept. of Microbiology, University of Washington, Seattle, WA 98195 U.S.A. (Tel) 206-653-8297

The MOLECULAR STRUCTURE AND REGULATION OF PHOTOSYNTHETIC PIGMENT SYSTEMS will be the topic of a satellite meeting, 27-30 Aug 1992, of the 9th International Congress on Photosynthesis. For information contact:

Mamoru Mimuro, National Institute for Basic Biology, Myodaiji, Okazaki, Aichi 444, JAPAN (Tel) 81-564-55-7514 (FAX) 81-564-53-7400 (EMail) Mimuro@Jpnonri.Bitnet

A SYMPOSIUM ON MICROBIAL ECOLOGY will be held in Barcelona, Spain, 6-11 Sept 1992. Contact:

Ricardo Guerrero, ISME-6, Apartado 16009, E-08080, Barcelona, SPAIN

The FIRST INTERNATIONAL SYMPOSIUM ON MEROMIXIS AND MICROSTRATIFICATION will be held the first week of September, 1992 in the Benedictine Monasterium of Banyoles, Spain, close to the Banyoles lake meromictic basins. It is anticipated that the proceedings of the Symposium will be published in one or another of the regular limnological series. Contact:

Carles Abella, Institute of Aquatic Ecology, Autonomous University of Barcelona, Hospital, 6 E-17071 Girona, SPAIN (FAX) 72-216406

The International Rice Research Institute has made available a booklet entitled "THE BLUE-GREEN ALGAE CULTURE COLLECTION AT IRRI". The collection comprises 204 unialgal strains originating from 21 countries. The strains were collected as part of a program to study the ecology of cyanobacteria and their possible use as biofertilizer in wetland rice culture. A complete description of the collection is available also in computer readable form, for those who have MacIntosh computers equiped with a hard disk and Hypercard 2 software (include two 4½ inch double sided diskettes with request). Requests should be addressed to:

Soil Microbiology Division, IRRI, Los Banos, Laguna, PHILIPPINES. Attention: Susan Ardales/P.A. Roger

POSITIONS AVAILABLE

CONTACT: Nancy Federspiel, Department of Bacteriology and Biochemistry, University of Idaho, Moscow, ID 83843 U.S.A. (Tel) 208-885-7481. (FAX) 208-885-5741. Send statement of interest, curriculum vitae, and three letters of reference.
RESEARCH: The ultimate goal of the research is to define the molecular componenets of the signal transduction pathway between the initial perception of light quality by cyanobacteria and the resultant changes in gene expression.
REQUIREMENTS: Ph.D. Experience in molecular biological and biochemical techniques.
START: As soon as possible.

CONTACT: F.K. Gleason, Department of Plant Biology, 220 BioScience Center, University of Minnesota, St. Paul, MN 55108 U.S.A. Send resume and names, addresses, and telephone numbers of three references.
RESEARCH: Conduct research on thioredoxins in cyanobacteria.
REQUIREMENTS: Degree in microbiology or biochemistry with experience in gene cloning, strain and vecotr construction, DNA sequencing, and site-directed mutagenesis. Some familiarity with protein purification, enzymology, or immunochemical techniques would be helpful but is not necessary.
SALARY: U.S. $21,800/year plus benefits, with funding available for a second year.
START: As soon as possible.

TRANSITIONS*TRANSITIONS*TRANSITIONS*TRANSITIONS*TRANSITIONS*TRANSITIONS*TRA

JEAN-MICHEL PANOFF has left his post-doctoral position in Peter Wolk's laboratory at Michigan State University and left the cyanobacterial world to study bacteria that make cheese. His new address:

Laboratoire de Génétique Microbienne, IRBA, Université de Caen, 14032 Caen Cédex, FRANCE (Tel) 33-31.45.55.00 (FAX) 33-31.45.56.00

DEVENDRA N. TIWARI has taken a short leave of absence from his laboratory in Varanasi to visit in the laboratory of Peter Wolk, where he will stay until November 30, 1991. He will study mutants of *Anabaena* defective in pattern formation. His current address:

MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824 U.S.A. (Tel) 517-353-6641 (FAX) 517-353-9168

NEWS*NEWS*NEWS*NEWS*NEWS*NEWS*NEWS*NEWS*NEWS*NEWS*NEWS*NEWS*NEWS*NEW

BOOSYA BUNNAG informs us that he and others at King Mongkut's Institute of Technology are involved in a collaborative effort with a private company for the cultivation of Spirulina in tapioca starch waste water. They are operating a 36 ton/month production plant. They are also interested in developing *Spirulina* as a source of γ-linolenic acid.

BIOENERGETICS AND BIOTECHNOLOGY WITH HALOTOLERANT CYANOBACTERIA

With all of eastern Europe looking towards the future, it is fitting that IGOR BROWN relates how he thinks the future looks for cyanobacterial research at Odessa University. His group has two primary focuses. First is the role of sodium in coupling photosynthetic processes. They recently showed that salt-resistant and salt-tolerant cyanobacteria in an alkaline medium carry out light-dependent extrusion of Na^+ from its cytoplasm against a concentration gradient. The resulting electrochemical Na^+ gradient across the cytoplasmic membrane serves as a source of energy for light-induced H^+ uptake and cyanobacterial movement. [Brown et al, FEBS Lett (1990) 270:203-206; Brown et al, Arch Microbiol (1990) 153:409-411]. Future work in this area is directed towards understanding the enzymatic mechanism by which light energy is converted into an electrochemical gradient of Na^+ ions. Specifically, they seek: (1) to identify enzymes operating as the primary Na^+-pumps in the cytoplasmic membranes, (2) to isolate these enzymes and reconstitute their activities in proteoliposomes, (3) to study the electrogenic properties of the enzymes within proteoliposomes, and (4) to determine the chain of events linking light absorption and generation of $\Delta\mu_{Na+}$ in salt- and alkalo-tolerant cyanobacteria.

The second focus is on biotechnological application of halo-resistant and halo-tolerant cyanobacteria, in particular, *Spirulina platensis*. Towards this end, they have gathered a collection of such cyanobacteria.

RESTRICTION/MODIFICATION SYSTEMS IN *NOSTOC* AND *PLECTONEMA*

M.I. MENDZHUL has sent in news about restriction-modification systems his group has found in two cyanobacteria. He, A.I. Melnik, and B.A. Rebentish have isolated and purified two type-two restriction endonucleases from the nitrogen-fixing cyanobacterium *Nostoc linkia*, isolated from a rice-field in the south of Ukraine. The molecular weight of the first restriction enzyme, called Nli387/7I, is 67500 Da, and that of the second, called Nli387/7II, is 65500. Maximum activity was found at 37° in 6 mM $MgCl_2$, 10 mM Tris-HCl, pH 9.0-9.5. Nli387/7I and Nli387/7II recognize same sequences as AvaI and AvaII, respectively. However, while AvaI cuts after the first base in the recognition sequence, Nli387/7I cuts after the fifth base, yielding a four-base 3' overhang.

Another member of his group, S.A. Sirchin, examined the state of methylation of DNA from *Plectonema boryanum*. HPLC analysis of hydrolyzed DNA from this organism showed the levels of N-6-methyladenine and 5-methylcytosine to be 4.5% and 1.2%, respectively. The specific sites of methylation was looked at further, using sets of restriction enzymes whose members are differentially sensitive to methylation of bases within the recognition sequence.

THE STATE OF CYANOBACTERIOLOGY IN ISRAEL

ELISHA TEL-OR has compiled summaries submitted by fellow cyanobacteriologists in ISRAEL and put together an overview of work that is being pursued in the area.

The Limnological Oceanic Institute in Eilat is a center for cyanobacterial activity. Amir Neori, Utsa Pollingher, Nehama Rushansky, and Tamar Zohary all have an active interest in the ecology of planktonic cyanobacteria. U.P. and N.R. have focused on phytoplankton of Lake Kinneret. T.Z. is interested in cyanobacterial hyperscums, food webs in Lake Kinneret, and microbial abundance and activity in the Eastern Mediterranean Sea. A.N. has a more southerly focus, studying micro- and nano-plankton populations in the Gulf of Aqaba.

Much of cyanobacterial research in Israel has a decidedly practical bent, particularly at Ben Gurion University of the Desert (BGU) in Sede Boker. There, Amos Richmond and Avigad Vonshak are both studying techniques of mass cultivation applicable to *Spirulina*. A.V. is also interested in the very related topics of how *Spirulina* responds to light stress and salinity stress. The latter topic is also being approached by Elisha Tel-Or at Hebrew University-Rehovot, especially with regard to osmoregulation and salt translocation. Zvi Cohen at BGU also has studied *Spirulina*. His particular interest is in using the cyanobacterium to overproduce γ-linelonic acid and other valuable chemicals. Arieh Zaritsky (BGU) is trying to use bacteria expressing cloned endotoxin genes from *Bacillus thuringiensis* var. israelensis as a means of controlling mosquito larvae. A.Z. has also worked with Samy Boussiba and Selwin Thomas on the rice-field cyanobacterium, *Anabaena siamensis*, hoping to improve its ability to provide rice with fixed nitrogen.

Two groups have adopted symbiotic cyanobacteria as their organisms of interest. Elisha Tel-Or's group studies carbohydrate metabolism of *Anabaena azollae*. Margalith Galun's group in Tel Aviv University also studies carbohydrates, but those on the outside of the cell. Her group focuses on the role of lectins in

establishing associations between cyanobacteria and plants, particularly lichen.

Photosynthesis is a popular topic in several institutes in Israel. Etana Padan (Hebrew University-Jerusalem) and Yosepha Shahak (Weizmann Institute, Rohovot) have a collaborative project, studying the sulfide-quinone reductase in the sulfide-dependent anoxygenic photosynthesic activity of *Oscillatoria limnetica*. Hadar Kless, also at the Weizmann Institute, prefers more conventional photosynthesis, devoting herself to study of the D1 protein. PSII occupies the thoughts of Devorah Friedberg (Hebrew University-Jerusalem) as well. Her angle is to seek mutants in the system.

The synthetic side of photosynthesis is well covered by several cyanobacteriologists. Aaron Kaplan (Hebrew University-Jerusalem) works on the CO_2-concentrating system within cyanobacteria. Both Michael Gurevitz (Tel Aviv University) and Devorah Friedberg are studying the *rbc* region, encoding (amongst other things) ribulose-bis-phosphate carboxylase. M.G. is attempting to genetically modify Rubisco, while D.F. is analyzing gene clusters around *rbcLS*.

Finally, there are those projects that don't fit neatly into any category. Doron Holland at the Volcani Institute, Bet-Dagan, is continuing his work on heterocyst differentiation in *Anabaena* PCC 7120, concentrating on the regulation of the *hetA* gene. Michael Gurevitz is interested in nitrogen regulation by non-nitrogen fixing cyanobacteria. Devorah Friedberg is working on the regulation of branched-chain amino acid biosynthesis. Elisha Tel-Or has an ongoing project concerning antioxidative mechanisms, with special reference to the role of ascorbate and ascorbate peroxidase in *Synechococcus* R2.

VII INTERNATIONAL SYMPOSIUM ON PHOTOSYNTHETIC PROKARYOTES

This year as in every year divisible by three with two left over, cyanobacteriologists the world over flocked to some common point to compare notes. The common point this time was Amherst, Massachusetts, USA, the site of the VII International Symposium on Photosynthetic Prokaryotes, held July 21-26. This was a wide-ranging meeting covering the ecology, systematics, biochemistry, and genetics of photosynthetic prokaryotes. Four contributors highlight below some of the presentations that stick in their minds. Necessarily, many interesting presentations, indeed, whole areas of research, have not been covered by the contributors.

Here are two non-scientific highlights. First, Urbino, Italy was chosen as the site of the next Symposium to be held in 1994. Second, the Belgium team of Dick Castenholz, Lucien Hoffmann, Andre Sobczyk, and Annick Wilmotte won the Official Symposium Turkey Shoot (i.e., basketball for the nonenergetic). The proceedings were enlivened by the appearance of a mystery team, sporting thick accents and claiming to be from some unknown duchy, that seemed invincible before they* were unmasked.

*(Lamont Anderson, Bob Kranz, Margaret Mechling, and Sandy Nierzwicki-Bauer)

NITROGEN HIGHLIGHTS

Genes encoding enzymes important in nitrogen assimilation: The two nitrogen sources alternative to ammonia most commonly used by the free-living cyanobacteria are nitrate and dinitrogen. In this Symposium, it was shown that a cluster of genes for nitrate assimilation is found in the genome of *Synechococcus* R2 (Omata & Andriesse, RIKEN, Saitama, and U.Utrecht; Flores et al., U.Sevilla), and Tatsuo Omata nicely demonstrated that genes encoding a multicomponent transport system for nitrate are present in that cluster. A new set of nitrogenase genes (*anf*), probably encoding a non-molybdenum, non-vanadium alternative nitrogenase, was shown to be present in *Anabaena* ATCC 29413 and some other heterocystous cyanobacteria, but not in *Anabaena* PCC 7120 (Thiel, U.Missouri, St.Louis).

Gene regulation by nitrogen availability: Ammonia behaves as a nutritional repressor both of enzymes for nitrate assimilation and of the whole machinery involved in the fixation of dinitrogen in cyanobacteria. A global nitrogen regulator, NtcA, which is required for the expression of proteins repressed by ammonia in the non-dinitrogen fixer *Synechococcus* R2, was shown to belong to the Crp family of transcriptional activators (Flores et al.). It is not known yet if NtcA controls nitrogen metabolism by itself or rather acts in concert with an Ntr-like system. Indications for the presence of an Ntr system in cyanobacteria were reported in the Symposium. The *glnB* gene encodes part of the nitrogen-sensing aparatus in enteric bacteria, and a similar gene has been found in many cyanobacteria (Tandeau de Marsac et al., Inst. Pasteur; Kaufman et al., Pennsylvania State U.). Furthermore, a gene similar to that which encodes the DNA-binding protein NtrB has been identified in *Calothrix* 7601 (Tandeau de Marsac et al.). So, we are just missing an NtrC-like protein

and a specialized sigma factor similar to NtrA! Such a sigma factor might be someday be found by two groups (Brahamsha & Haselkorn, U.Chicago; Caslake & Bryant, Pennsylvania State U.) that are characterizing cyanobacterial RNA polymerase, with special emphasis on its sigma factors.

Heterocyst differentiation: Removal of combined-nitrogen from the medium initiates the process of development of heterocysts in certain nitrogen-fixing cyanobacteria. Peter Wolk (Michigan State U.) described the use of a modified version of Tn*5* that carries promoterless *luxAB* genes (encoding luciferase), which serves to place transcriptional reporters at many different positions in the chromosome. We heard from Bob Haselkorn (U.Chicago) an alternative strategy to identify early genes involved in the process of differentiation, based on the isolation of cDNA libraries specific to different developmental stages.

A number of early genes involved in heterocyst development have been mutated, by chemical mutagenesis and by using transposon mutagenesis to alter the expression of a *hetA::lux* fusion (*hetA* is a gene encoding a product essential for the normal biosynthesis of the heterocyst cell wall). Bill Buikema (U.Chicago) summarized work on one particularly interesting gene, *hetR*, that encodes a product that is an essential regulator of heterocyst development. The expression of *hetR* is increased in response to nitrogen starvation (Buikema & Haselkorn), and studies with a *hetR::lux* fusion suggested that *hetR* is autorepressible (Black & Wolk). Some mutants altered in the spacing of heterocysts were also reported (Liang & Haselkorn). One of these mutants develops only terminal heterocysts and suppresses the ability of plasmid-borne *hetR* to elicit supernumary heterocysts. The availability of new genetic tools and the cloning of some genes involved in development suggests that there may be rapid progress in the near future in our understanding of the genetic control over heterocyst differentiation.

Regulation of *nif* gene expression by anaerobiosis: It is clear that in aerobic cultures, the expression of *nifHDK* (encoding nitrogenase) is restricted to heterocysts, the site of active nitrogenase. But does *nif* gene expression respond to microaerobic conditions within the heterocyst or is it a final step within the developmental program? Published work has suggested a developmental control over *nif* gene expression in heterocystous cyanobacteria. However, Sandy Nierzwicki-Bauer and Anu Madan (Rensselaer Polytech.Inst.) in this Symposium reported that under strict anaerobic conditions *nif* transcripts were detected in all of the cells of filaments of *Anabaena* PCC 7120. Furthermore, we learned that expression of *nifH** requires strict anaerobiosis in addition to removal of combined nitrogen (Cannell & Robinson, U.Massachusetts). So, is there a different regulatory pathway for *nif* gene expression operating under strict anaerobic conditions? Stephen Murphy and Martin Mulligan (Memorial U.,Newfoundland) are attempting to determine a consensus cyanobacterial *nif* promoter by comparison of a number of *nif* promoters from several heterocystous cyanobacteria.

What level of oxygen is required to influence genetic events within heterocysts? Jeff Elhai and Peter Wolk showed that the *nifHDK* promoter is expressed in a *hetA* mutant of *Anabaena* PCC 7120 under aerobic conditions that prevent nitrogen fixation. In addition, Peter Lammers and Alexander Ryncarz II (New Mexico State U.) showed that in another mutant of PCC 7120 defective in the heterocyst envelope and aerobic nitrogen fixation, excision of the *nifD* and *nifS* elements also takes place under aerobic conditions. Therefore, at least in *Anabaena* PCC 7120, oxygen levels high enough to prevent function of nitrogenase do not repress *nif* gene rearrangements or expression.

Let's hope that for the next Photosynthetic Prokaryotes Symposium we are able to put together all of those proteins that can participate in global nitrogen control, get a clear view of the triggering of the process of heterocyst development, and find what genes are expressed during that developmental process and how their stepwise expression is coordinated.

-- Enrique Flores

PHYCOBILISOME HIGHLIGHTS

Chromophore biosynthesis and attachment: The covalent attachment of bilin chromophores to the apo-biliproteins presents an interesting problem in molecular recognition. Some isologous chromophore sites on different classes of biliproteins are nearly identical in primary structure while supporting the specific attachment of either phycoerythrobilin or phycocyanobilin chromophores. Specificity for proper chromophore attachment may reside in accessory proteins and Craig Fairchild and Alex Glazer (U.California, Berkeley) are using an in vitro approach to isolate such factors. They have coupled apo-α-phycocyanin (obtained from

E. coli) to an affinity resin and demonstrated proper chromophore attachment to the protein when the resin is treated with cell extracts of *Synechococcus* PCC 7002. This contrasts with the spontaneous in vitro attachment of purified phycocyanobilin to apo-α-phycocyanin, which produces an improper adduct. Their poster suggests that Craig and Alex are close to isolating the cell extract factor(s) that promote proper chromophore attachment in vitro.

Sam Beale (Brown University) has been investigating the bilin biosynthesis pathway in a biliprotein-synthesizing eucaryote, *Cyanidium caldarium*, using biochemical and NMR methods to isolate and identify possible intermediates in the pathway. The earlier steps in the pathway from protoheme to phycocyanobilin have been well characterized by Sam, but I find the most interesting observation to be the presentation of phycoerythrobilin as a possible intermediate for phycocyanobilin, and this in an organism that does not synthesize phycoerythrin. If this situation exists in cyanobacteria, it may be possible to study differential chromophore attachment between phycocyanin and phycoerythrin in vivo. This would require the transfer of genes for phycoerythrin and its putative chromophore attachment factors into a transformable cyanobacterium (no phycoerythrin-synthesizing cyanobacterium has been shown to be competent for gene uptake).

Structure, assembly and function: The general structural features of the hemi-discoidal phycobilisomes found in many cyanobacteria have been well characterized due to their distinct appearance in the electron microscope. Detailed structural models for the hemi-spherical phycobilisomes found in some red algae have not been constructed because the large number of rods that converge at the core in these phycobilisomes results in very little structural information at the rod-core junction in the electron microscope. Manuel Glauser (and others at ETH, Zürich, and Pennsylvania State U.) presented a poster on phycobilisomes from *Mastigocladus* and *Anabaena* sp. showing the presence of multiple rod-core linker proteins. Their model incorporates different species of rod-core linkers into different rod sub-structures with variation in composition (including allophycocyanin in a rod) and attachment site. This increased complexity of phycobilisome architecture suggests the structural solution to jamming as many rods as possible around a single phycobilisome core, as appears to be the case in the oblate, hemi-spherical phycobilisomes of red algae.

Véronique Capuano (and others at Inst.Pasteur) presented a model for the interaction of the "anchor" protein with the various biliproteins complexes in the phycobilisome core. It is based upon the presence of repeat regions that have structural homology with rod-linker proteins and that are assumed to be the basis of "anchor"-biliprotein interactions. Phycobilisomes with more complex cores have a larger number of these repeats in the "anchor" amino acid sequence. Perhaps we should consider calling this a "scaffold" rather than an anchor. On the other hand, the "anchor" appellation would be a small sacrifice to tradition, and the model still includes a membrane-associated loop for this protein.

Jianhui Zhou and Don Bryant (Pennsylvania State U.) presented a very complete study on the terminal energy transfer steps in phycobilisomes from *Synechococcus* PCC 7002. A combination of insertional inactivation, mutagenesis, gene replacement, and fluorescence energy transfer and growth measurements resulted in conclusions that the allophycocyanin-B α subunit chromophore is at most a minor partner in energy transfer to chlorophyll, that the "anchor" protein chromophore is the major energy conduit for photosynthesis. While the notion is not new, this is the first genetic/functional proof of terminal transfer roles in cyanobacterial phycobilisomes.

Finally, a long mysterious protein in phycobilisome preparations appears to have been identified. The "45kd" protein has been described as stoichiometric, substoichiometric, heterogenous, variable, and non-existent, in response to light quality, all depending upon the species, lab, or condition of phycobilisome isolation. Wendy Schluchter and Don Bryant have isolated the gene for ferredoxin-$NADP^+$ oxidoreductase (FNR) from *Synechococcus* PCC 7002 and shown that this protein has a cytosolic form and an acylated, presumably membrane-bound form. The 110 amino acid N-terminal of this FNR is 78% homologous to a 9 kDa phycobilisome protein that interacts with phycocyanin. Is this homology the product of a functional association of FNR with the cyanobacterial phycobilisome, or does it simply provide a fortuitous mechanism for co-isolation?

Light regulated gene expression in chromatic adaptation: *Calothrix* PCC 7601 is fast becoming the best characterized cyanobacterium for chromatic adaptation studies. Jean Houmard, Andre Sobczyk, and Nicole Tandeau de Marsac (Inst. Pasteur) isolated a cell extract fraction that contained a factor that binds to a DNA region upstream of the light-regulated phycoerythrin transcript. Treatment with phosaphatase abolishes the DNA-binding capacity of this fraction. An in vitro footprint analysis of binding showed protection of a 20 bp region that includes two hexameric repeats, TTGTTA, that are also present upstream of the light-regulated phycoerythrin genes from *Synechocystis* PCC 6701. Nancy Federspiel (U. Idaho), also working with *Calothrix*, showed in vivo footprint analysis with dimethylsulfate that confirmed the protection of the G residues within

the hexameric repeats. Characterization of these DNA binding factors in the near future will provide the first steps in tracing the signal transduction pathway back towards the light signal and the photoreceptor that control the chromatic adaptation process.

-- Lamont Anderson

PHOTOSYNTHESIS HIGHLIGHTS

Photoinhibition: Wim Vermaas (Arizona State U.) reported progress in studying photoinactivation of oxygen evolution in the *Synechocystis* PCC 6803 D2 mutants E69Q, P161L and G215W. These three mutants are incapable of photoautotrophic growth but are able to evolve oxygen for short periods ($t_{½}$=80-100 sec). Inactivation of oxygen evolution is accelerated under high light intensities. W.V. proposed that the primary site of damage during photoinhibition occurs on the donor side of Photosystem II rather than the acceptor side. Revertants from the E69Q mutant which are able to grow photoautotrophically but which are not E69Q to E or D reversions are being investigated. In one particularly interesting photosynthetic revertant, the secondary lesion appears to be in a gene other than *psbA* or *psbD*. Steve Mayes also reported that the Imperial College group has found that *Synechocystis* PCC 6803 *psbO* mutants, whilst still capable of photoautotrophic growth, show an increased vulnerability to photoinhibition.

A poster from Norio Murata's group (NIBB, Okazaki) described further work on Fad6 and Fad12, mutants of *Synechocystis* PCC 6803 defective in desaturation. These mutants are, respectively, unable to introduce double bonds into the Δ6 and Δ12 positions of C_{18} fatty acids of membrane lipids. The *desA* gene, which complements Fad12 to a wild-type phenotype, was interrupted in vitro with a kanamycin resistance cassette and transformed into Fad6 to generate the double mutant Fad6/*desA*::Km^r. Fatty acids from this strain were not desaturated in either the Δ6 or Δ12 positions. Significantly it was found that the double mutant exhibited an increased vulnerability to photoinhibition (assayed by measuring the rate of oxygen evolution), particularly at lower temperatures. However the heat-induced inactivation of photosynthesis was unchanged in the double mutant compared to the wild-type.

Photosystem II (PSII): Lou Sherman (Purdue U.) reported thermoluminesence results that indicate that the S2 to S3 transition rate is decreased in a mutant strain of *Synechocystis* PCC 6803 in which *psbO* has been deleted. Additionally, the *psbO* point mutant D9N apparently does not bind well to PSII cores in vivo whilst the mutant D9K interacts with PSII much better.

Posters from Wim Vermaas' group showed that small deletions in the region predicted to form the large hydrophilic lumenal loop between helices V and VI of CP-43 all result in *Synechocystis* PCC 6803 mutants that are obligate photoheterotrophs. In one case, replacing the deleted amino acids with a randomly generated sequence of similar length restored photoautotrophic growth, suggesting that the length of the loop region may be important for correct functioning. However, in the corresponding loop region of CP-47, small loop segments can be deleted without major functional disturbances. Study of PSII was facilitated by a new protocol for preparing functional PSII particles from *Synechocystis* PCC 6803, using dodecyl-B-D-maltoside and octyl-B-D-glucoside extraction.

Mysterious herbicide resistance: Sergei Shestakov's group (Moscow State U.) has complemented mutants of *Synechocystis* PCC 6803 resistant to the phenolic herbicide dinoseb. The partial sequence of the gene responsible for complimentation was presented and showed no homology to any known photosynthetic gene. In addition the mutant, SK18, cannot grow photoautotrophically yet is capable of oxygen evolution. This mutant has been complimented by gene *psX* which hybridises to plant nuclear DNA. A partial sequence of *psX* was presented.

Chantal Astier (CNRS Gif-sur-Yvette) presented preliminary data on an intriguing metribuzin-resistant *Synechocystis* PCC 6714 mutant, strain M30, in which the lesion is neither in the D1 protein Q_B-binding region nor in D2. Flash studies show that maximal oxygen evolution occurs on the fourth rather than the third flash following dark adaptation.

Photosystem I (PSI): Don Bryant (Pennsylvania State U.) described experiments where the *psaD*, *psaE* and *psaC* genes were expressed in *E. coli*, the proteins purified and successfully reconstituted into *Synechococcus* PCC 6301 cores. EPR spectroscopy indicated functional electron transfer to the Fe-S centres F_A and F_B. Site-directed mutagenesis on the Cys residues in the PsaC protein are in progress. Himadri Pakrasi (Washington U.,St.Louis) reported progress in the use of *Anabaena variabilis* ATCC 29413, which can grow

heterotrophically in the dark on media supplemented with fructose, for targeted mutagenesis of PSI genes. Mutants with lesions in *psaA*, *psaB*, *psaC*, and *psaI* have been constructed and phenotypic characterisation is currently in progress.

Gene expression: Sue Golden (Texas A&M) updated her lab's work on the differential gene expression of the *psbA* and *psbD* gene families in *Synechococcus* PCC 7942, encoding, respectively, D1 and D2 proteins of the PSII reaction centre. The gene *psbAI* encodes form I of D1 and is primarily expressed in low light regimes, whilst a shift to higher light results in the predominant expression of form II of D1 from the *psbAII* and *psbAIII* genes. A factor specifically involved in the degradation of the *psbAI* transcript is synthesized under high light conditions. The expression of the monocistronic *psbDII* gene increases at higher light intensities. This possibly represents a mechanism to compensate for the increased turnover of the PSII D2 polypeptide with repect to CP-43 in high light conditions, in an interactive manner with the dicistronic *psbDI-psbC* operon. In competition experiments, *psbDII*$^-$ mutants showed decreased viabilities with respect to the wild-type when plated at high light intensities.

Stephanie Curtis described work on promoter characterisation in *Anabaena* PCC 7120. Alignment of promoter regions for the genes *rbcL*, *atp1*, *atp2*, *petF1*, *psbAI*, *psbAII*, *psbAIII*, and *psbAIV* revealed a consensus sequence reasonably similar to *E. coli* "-10" promoter regions but no real conservation in the "-35" region. Reporter constructs where different lengths of upstream sequence were fused to the CAT reporter gene have been used to assay promoter strength and define the promoter elements. Preliminary results indicate that only relatively small upstream regions are needed for accurate and abundant initiation of transcription.

I would like to thank Clint Fuller and all our U. Massachusetts hosts for organising such a thoroughly enjoyable meeting. Thanks for looking after us so well! In particular, the Clam Bake (thanks to Jeff Trost for the Sam Adams when I pulled the old penniless trick!) where New England lobster, chowder and clams were dished up, lives in the memory.

-- Steve Mayes

MOLECULAR BIOLOGY HIGHLIGHTS

Heterocyst differentiation: At least two DNA rearrangments take place during heterocyst differentiation in *Anabaena* PCC 7120: an 11 kb element is excised from within the *nifD* gene, and a 55 kb fragment is excised from within the *fdxN* gene. An essential gene for the first event is *xisA*. Claudio Carrasco and others at Texas A&M U. appear to have cloned an essential gene, *xisF*, for the second event. The same group has isolated two different protein factors (VF1 and VF2) that bind to AT-rich DNA sequences upstream of *xisA* (Ramasubramanian et al).

Light regulation: DNA-binding proteins figure prominantly in reports in this area as well. Susan Golden (Texas A&M) reported on gel retardation experiments with promoter-containing DNA that reveal three protein binding sites exist near the translational start of *psbDII*. DNA from the promoter regions of *psbDII* and *psbAII* compete for the same factor. Proteins proteins that bind to the promoter region of the *cpeBA* operon were also isolated from *Calothrix* PCC 7601 (also called *Fremyella diplosiphon*) grown under green light [also discussed above by Steve Mayes]. John Cobley's group (U.San Francisco) isolated a region of DNA from the same organism which, when introduced on a plasmid, causes an increase in phycoerythrin synthesis in green light. The region contains an open reading frame capable of encoding a polypeptide with similarity to a DNA-binding protein and a yeast enzyme involved in the synthesis of ubiquinol.

Protein transport: Cyanobacteria may be useful as a model system for studying protein sorting in chloroplasts among different membrane systems. Dirk Geerts and others at U.Utrecht showed that a cyanobacterial signal peptide from *petE* (encoding plastocyanin) of *Anabaena* is functional in the chloroplast, both in its ability to route protein to the thylakoid membrane as well as its ability to be recognized by thylakoidal processing peptidases. When the *petE* gene of *Anabaena* was expressed in *Synechococcus* PCC 7902 (which lacks endogenous plastocyanin) under the control of the *E.coli trc* promoter, plastocyanin was targeted to the thylakoid membrane as well as the periplasm. D.G. et al. are trying to isolate genes involved in protein transport by the following strategy. The expression of fructosyltransferase (FTF) of *Streptococcus* in *Synechococcus* PCC 7902 is lethal when sucrose is present in the medium, so long as FTF is targeted to the

periplasm. Mutants that have gained resistance to sucrose, and which therefore may lack periplasmic FTF, are currently under study.

-- Mies Borrias

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